Analysis of Bioplex Syphilis IgG Quantitative Results in Different Patient Populations$^\triangledown$

Because of the need to reduce labor costs, many laboratories are replacing the traditional syphilis testing algorithm—screening with a manual nontreponemal test, followed by an anti-*Treponema pallidum* antibody test—with a "reverse" algorithm that uses an automated immunoassay to screen for anti-*T. pallidum* IgG antibodies. A rapid plasma reagin (RPR) titer determination is then performed on IgG-reactive specimens to (i) verify syphilis by an alternative method and (ii) obtain a titer for patient management. In addition, some laboratories also perform a traditional treponemal assay (fluorescent treponemal antibody absorbed [FTA-ABS] or *T. pallidum* particle agglutination [TP-PA] assay) on specimens that test positive by an IgG screening assay. A number of studies have shown that anti-*T. pallidum* IgG immunoassays have sensitivities and specificities that are comparable to those of other treponemal assays and nontreponemal assays (1, 3, 4). As with other highly sensitive screening tests, anti-*T. pallidum* IgG immunoassays can generate false-positive results, with a lower positive predictive value in low-prevalence populations (2). Yen-Lieberman et al. recently reported that the strength of signal (antibody index [AI]) of the Bioplex 2200 syphilis IgG multiplex flow immunoassay (Bio-Rad Laboratories, Hercules, CA) could be used to identify likely false-positive results and thereby reduce the need for confirmatory testing (5). They demonstrated that specimens with Bioplex AIs of ≥6.0 were always positive when tested with a supplemental enzyme immunoassay (EIA) and therefore proposed an algorithm in which only specimens with a Bioplex syphilis IgG AI of <6 are subjected to confirmatory EIA. Their study was performed on specimens from a low-prevalence population but did not describe specific population characteristics. In order to further verify the efficacy of the use of the quantitative Bioplex syphilis IgG data, we evaluated AI results from three different patient cohorts (incarcerated individuals, women attending obstetrics and gynecology [OB/Gyn] clinics, and women at delivery) for their ability to predict TP-PA results. Data were stratified by RPR test result. This study was approved by the University of Texas Medical Branch (UTMB) Institutional Review Board.

We performed a retrospective review of test results and patient data from the UTMB laboratory information system for serum specimens submitted for routine syphilis testing during December 2010 and January 2011. A total of 1,849, 3,512, and 873 specimens were linked to incarcerated individuals, women attending UTMB clinics for prenatal or gynecological care, and women at delivery, respectively. Among the incarcerated individuals, over 96% of the specimens were from men. The distribution of specimens by individual race or ethnicity was as follows: Hispanic, 49.4%; African-American, 27%; white/non-Hispanic, 21.4%; and other/unknown, 2.2%. Among the OB/Gyn patients, the distribution of specimens by individual race or ethnicity was as follows: Hispanic, 63.1%; white/non-Hispanic, 21.8%; African-American, 12.6%; and other/unknown, 2.5%. Among women delivering at UTMB hospital, the distribution of specimens by individual race or ethnicity was as follows: Hispanic, 71.8%; white/non-Hispanic, 15.8%; African-American, 10%; and other/unknown, 2.4%.

The Bioplex 2200 syphilis IgG, RPR (Sure-Vue; Biokit USA, Inc., Lexington, MA), and TP-PA (Fujirebio, Inc., Tokyo, Japan) assays were performed according to the instructions of the manufacturers. The Bioplex 2200 syphilis IgG assay has been cleared by the U.S. Food and Drug Administration for use as a qualitative assay. Initially, specimens with results that were equivocal (AI = 0.9 to 1.0) or reactive (AI ≥ 1.1) by Bioplex were tested by semiquantitative RPR only. In cases in which the RPR test was nonreactive, a TP-PA assay was performed. Beginning in the middle of December 2010, all specimens that were equivocal or reactive by Bioplex were tested by both semiquantitative RPR and TP-PA assays. The sensitivity and specificity of Bioplex, based on an AI cutoff value of either 6 or 8, were calculated using 2-by-2 contingency tables. The TP-PA assay was considered the reference method. Receiver operating characteristic (ROC) analysis was performed using the web-based calculator available at http://www.rad.jhmi.edu/jeng/javarad/roc/JROCFITi.html (accessed 5 July 2011).

The anti-*T. pallidum* IgG-reactive rates determined by the Bioplex assay for the incarcerated, OB/Gyn, and delivery cohorts were 7.5%, 1.6%, and 2.6%, respectively (data not shown). The ability of the AI value of the Bioplex IgG assay to predict a reactive TP-PA result varied by patient cohort and by the accompanying RPR titer (Table 1). As reported by Yen-Lieberman et al. (5), specimens that exhibited reactive RPR titers were more likely to exhibit reactive TP-PA results. However, we evaluated specimens with an RPR titer of 1:1 separately from those with titers of ≥2 because of clinical and epidemiological evidence indicating that an RPR titer of 1:1 is not always sufficient to confirm a reactive IgG result. Indeed, our data show that IgG-reactive specimens with RPR titers of ≥2 are more likely to be confirmed by the TP-PA assay. Among the OB/Gyn and delivery cohorts, there were no TP-PA-nonreactive results when the RPR titer was ≥2. Among the incarcerated and OB/Gyn cohorts, an AI cutoff of 8 provided higher specificity than a cutoff of 6. Yen-Lieberman et al. showed that an AI of 6 provided 100% specificity for their study population (5). Even at a cutoff of 8, the specificity of the Bioplex IgG assay in our study failed to reach 100% in the incarcerated cohort for specimens with RPR-nonreactive results or with a titer of 1:1 (often considered an equivocal titer). This cohort is at high risk for syphilis, with an IgG-reactive rate of 7.5%. Among the specimens from incarcerated individuals, 85.2% (98/115) of the IgG-reactive specimens were confirmed by the TP-PA assay (data not shown). In comparison, the IgG reactivity rate in the OB/Gyn cohort was 1.6%; 63.0% (29/46) of the results were confirmed by the TP-PA assay. To assess the effect of the Bioplex syphilis IgG AI cutoff values on the sensitivity and specificity of the assays, a ROC analysis was performed. The ROC analysis showed that the specificity of the syphilis IgG assay for identifying TP-PA-confirmed samples usually declined rapidly at AI values below 8.

In conclusion, we demonstrate for the first time the utility of quantitative Bioplex syphilis IgG data in different patient populations and the usefulness of a cutoff value to identify specimens that may not require an additional treponemal assay for confirmation. An AI cutoff of 8 was necessary to achieve maximum specificity in our incarcerated and OB/Gyn cohorts. RPR titers of ≥2 are sufficient to suggest that a reactive Bio-

TABLE 1. Bioplex syphilis IgG performance based on user-defined cutoff values in different patient cohorts[a]

Patient cohort	AI cutoff	No. of specimens with indicated Bioplex result/TP-PA result				Sensitivity (%)	Specificity (%)	ROC AUC (SE)
		+/+	−[b]/−	−/+	+/−			
RPR nonreactive								
Incarcerated	≥6	33	23	24	2	33/57 (57.9)	23/25 (92)	0.832 (0.047)
	≥8	29	24	28	1	29/57 (50.9)	24/25 (96)	
Women attending OB/Gyn clinics	≥6	11	21	10	2	11/21 (52.4)	21/23 (91.3)	0.956 (0.035)
	≥8	11	23	10	0	11/21 (52.4)	23/23 (100)	
Women at delivery	≥6	1	9	5	0	1/6 (16.7%)	9/9 (100)	0.763 (0.134)
	≥8	1	9	5	0	1/6 (16.7%)	9/9 (100)	
RPR titer = 1:1								
Incarcerated	≥6	17	2	5	2	17/22 (77.3)	2/4 (50)	0.803 (0.083)
	≥8	17	3	5	1	17/22 (77.3)	3/4 (75)	
Women attending OB/Gyn clinics	≥6	3	5	2	1	3/5 (60)	5/6 (83.3)	0.894 (0.098)
	≥8	2	6	3	0	2/5 (40)	6/6 (100)	
Women at delivery	≥6	3	2	2	0	3/5 (60)	2/2 (100)	0.915 (0.11)
	≥8	3	2	2	0	3/5 (60)	2/2 (100)	
RPR titer ≥ 1:2								
Incarcerated	≥6	106	2	3	0	106/109 (97.2)	2/2 (100)	0.986 (0.011)
	≥8	103	2	6	0	103/109 (94.5)	2/2 (100)	
Women attending OB/Gyn clinics	≥6	30	0	1	0	30/31 (96.8)	N/A	N/A
	≥8	30	0	1	0	30/31 (96.8)	N/A	
Women at delivery	≥6	7	0	0	0	7/7 (100)	N/A	N/A
	≥8	7	0	0	0	7/7 (100)	N/A	

[a] Data represent all specimens reactive by Bioplex 2200 syphilis IgG assay (antibody index ≥ 1.1). RPR, rapid plasma reagin; AI, Bioplex IgG antibody index; TP-PA, *Treponema pallidum* particle agglutination; ROC AUC, receiver operating characteristic area under the curve; N/A, not applicable.
[b] −, nonreactive or inconclusive.

plex IgG result in both low- and high-risk cohorts represents an accurate determination of exposure to *T. pallidum*. Finally, laboratories that choose to use the AI value as part of their syphilis IgG test must independently verify the performance characteristics of this modification to the manufacturer's recommended procedure. This is also important because, as we have shown, the optimal AI cutoff may vary depending on the patient population.

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